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Elektronenmicroscopie van ferritine en hemocyanine

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Summary

ELECTRON MICROSCOPY OF FERRITIN AND HEMOCYANIN

1. INTRODUCTION

The concepts primary, secondary, tertiary and quarternary structure of proteins are described. Electron microscopy chiefly deals with the quarternary structure: the way protein molecules are built from subunits.

By electron microscopy we have studied the structural changes of ferritin and hemocyanin molecules under the influence of different chemical agents.

2. TECHNIQUES

The preparation of carbon films and the way protein molecules are sprayed onto the carbon film are described. The various staining methods for proteins can be divided in shadow-techniques, positive- and negative-staining methods. Several negative-staining agents are discussed e.g. potassium phosphotungstate (PTA), sodium tungstate, uranyl acetate and a complex of uranyl acetate with ethylene diamine tetraäcetate ("uranyl. EDTA-complex").

Use was made of the Siemens Elmiskop-I and the Philips EM-100 and EM-200 electron microscopes. The beam voltages were between 40 and 80 kV, the "spot" size was 15-25 μ, the emission current about 20 μA and the diameter of the objective aperture 25-50 μ. The astigmatism of the objective lens was reduced to less then 0.2 μ.

Photographs were taken on Ilford 5B11 35 mm film at a magnification of 18,000-35,000 x and on Ilford Special Lantern Plates (gradation: contrasty) at a magnification of 40,000-80,000 x. The films were developed in a ten times diluted Glycinool developer (make: Amaloco), the plates in Kodak D61A developer for 15 and 3 minutes respectively. The developing temperature was 18-20° C.

3. FERRITIN

Ferritin is a remarkable protein, because of its high iron content (about 20 % of its dry weight). The iron is concentrated in a micelle of composition $(FeO.OH)_8(FeO.OPO_3H_2)$. When the iron is removed from ferritin by the action of reducing agents *apoferritin* is formed. It is possible to introduce some iron again into apoferritin, by which a *"synthetic ferritin"* is obtained.

A short survey is given of the literature on ferritin. It was shown previously by electron microscopy that a ferritin molecule consists of the iron containing micelle surrounded by an approximately spherical shell of apoferritin.

We studied the structure of the ferritin molecule in some detail, the intermediate stages of the molecules during the removal of the iron, the apoferritin molecule and the molecule of "synthetic ferritin". Use was made of the replica and shadow-technique of HALL and several negative-staining methods.

A model is given for the iron-containing *micelle;* it consists of 6 subunits at the corners of a *trilateral prism* (pointgroup symmetry $\bar{6}$). The structure of *apoferritin* could not be elucidated, although an indication of the arrangement of the subunits has been obtained. The centre of the apoferritin molecule is hollow and filled with the surrounding solution.

In Table III the Fe/N-weight ratios of ferritin, apoferritin and the intermediate stages are given. The proportions of the number of full, partly full and empty molecules as observed on electron micrographs are listed for each Fe/N-ratio. The results are also shown in Figure I. It is concluded that the removal of iron from ferritin is a process with an *"all or none"* character.

Electron micrographs of *"synthetic ferritin"* show that the iron-containing micelles in the centres of the molecules have been regenerated to a certain extent. About one half of the apoferritin molecules is partly filled up with "iron hydroxide". Complete refilling does not take place, probably because of denaturation of the apoferritin molecules during removal of the iron.

4. HEMOCYANIN

Hemocyanins are oxygen-transporting, copper-containing blood

proteins occurring freely dissolved in the blood of several invertebrates as crabs, squids, snails and scorpions. The molecular weights of the undissociated molecules range from about 800,000 to about 8,000,000 depending on the biological origin. The molecules can be dissociated reversibly into smaller units by changing the pH or the kind and the concentration of the salts present in the solution.

A survey is given of the literature on hemocyanin. Our own work mainly deals with:

1. The structural relationship between the undissociated molecules and the dissociation products of *Helix pomatia* (roman snail) and *Octopus vulgaris* (a squid) hemocyanins.
2. A possible structural correspondence between the undissociated hemocyanin molecules of different biological origin. We compared the hemocyanins of *Helix pomatia, Octopus vulgaris, Sepia officinalis* (another squid) and *Cancer pagurus* (a crab).

Electron micrographs were taken from *Helix pomatia* hemocyanin at different pH values with the negative-staining method. The dissociation was studied as a function of pH at both sides of the isoëlectric point. Our observations are in agreement with results reported previously by other authors, although they are not compatible with the model and mechanism of dissociation proposed previously. The electron micrographs suggest that the *Helix pomatia* hemocyanin molecule is roughly *cylindrical;* the diameter of the cylinder is about 300 Å and the height is about 335 Å. This cylinder consists of a stack of 6 parallel plates of subunits and has a five-fold axis. The first dissociation step occurs perpendicular to the five-fold axis into halves. Further dissociation is supposed to be a sectorwise splitting of these flat cylinders via submultiples into subunits. *Divalent cations* in concentrations of about 10^{-4} molar or higher are required for the reversibility of the dissociation.

Preliminary results indicate a similar behaviour for *Helix pomatia apo*hemocyanin.

Electron micrographs of *Octopus vulgaris* and *Sepia officinalis* hemocyanins show the molecules in the region of the isoëlectric point as *flat cylinders* consisting of 3 parallel layers perpendicular to a five-fold axis. These cylinders have a height of about 140 Å and

a diameter of about 300 Å. They closely resemble the half-molecules of *Helix pomatia* hemocyanin, although, amongst others, differences are observed in the arrangement of the subunits.

The dissociation as a function of pH was preliminarily studied at both sides of the isoëlectric point. The influence of divalent cations on this dissociation was not investigated.

Cancer pagurus hemocyanin consists of smaller molecules, which are tentatively represented as a *pentalateral prism.*

The undissociated molecules of *Helix pomatia, Sepia officinalis, Octopus vulgaris* and probably those of *Cancer pagurus* have their cylindrical (prismatical) layer structures in common. These layers are perpendicular to the cylinder axis, which is an axis of five-fold symmetry.

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